Quick-Start Protocol April 2016

# EpiTect® Fast 96 Bisulfite Kit – Part 1

DNA Protect Buffer and Buffer BD from the EpiTect Fast 96 Bisulfite Kit (cat. no. 59720) should be stored at 2–8°C. Bisulfite Solution and all other buffers should be stored at room temperature (15–25°C) for up to 6 months if not otherwise stated on label.

## Further information

- *EpiTect Fast 96 Bisulfite Conversion Handbook*: **www.qiagen.com/HB-1212**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- Add 120 ml ethanol (96–100%) to Buffer AW2 and store at room temperature.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C.
- Add 1350 µl RNase-free water to carrier RNA and store in aliquots at –20°C.
- Equilibrate samples and buffers to room temperature.

## Bisulfite conversion of DNA

1. Thaw DNA. Completely dissolve the Bisulfite Solution. If necessary, heat the solution to 60°C and vortex until all precipitates are dissolved.
2. Prepare the bisulfite reactions in the EpiTect 96 Conversion Plate according to Table 1. Add each component in the order listed.

   **Note**: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect 96 DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.
3. Seal the EpiTect 96 Conversion Plate using EpiTect 96 Cover Foil and mix by vortexing. Centrifuge the plate briefly at 650 x *g* at room temperature.

   **Note**: The buffer turns blue, indicating sufficient mixing and correct pH.

**Note**: If the thermal cycler is not compatible with EpiTect 96 Cover Foil, cap strips can be used.

**Table 1. Bisulfite reaction setup**

| | Volume per reaction, µl | |
|---|---|---|
| **Component** | **High concentration samples (1 ng – 2 µg)** | **Low concentration samples (1–500 ng)** |
| DNA | Variable* (maximum 20 µl) | Variable† (maximum 40 µl) |
| RNase-free water | Variable* | Variable† |
| Bisulfite Solution | 85 | 85 |
| DNA Protect Buffer | 35 | 15 |
| **Total volume** | **140** | **140** |

* The combined volume of DNA solution and RNase-free water must total 20 µl.

† The combined volume of DNA solution and RNase-free water must total 40 µl.

4. Program the thermal cycler according to Table 2. Use a cycler with a heated lid.

   **Note**: If using a thermal cycler that does not allow you to enter the reaction volume (140 µl), set the instrument to the largest volume setting available.

**Table 2. Bisulfite conversion thermal cycler conditions**

| Step | Time | Temperature |
|---|---|---|
| Denaturation | 5 min | 95°C |
| Incubation | 10 min‡ | 60°C |
| Denaturation | 5 min | 95°C |
| Incubation | 10 min‡ | 60°C |
| Hold | Indefinite§ | 20°C |

‡ In some cases it may be necessary to extend the 60°C cycle time up to 20 min to achieve complete bisulfite DNA conversion.

§ Converted DNA can be left in the thermal cycler overnight without any loss of performance.

5. Place the plate in the thermal cycler and start the incubation.

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